

transplant rejection (also termed host-versus-graft reaction), in which the endogenous immune system reacts against, causing the destruction of, foreign tissue. Tissue rejection can be mediated by humoral and cellular immune responses. In the case of genetically modified cells generated for the purpose of incorporating a missing copy of a gene (gene therapy) or to help the patient eliminating cancerous cells (e.g., CAR-T therapies), there is a risk that some of the "machinery" utilized for the genetic modification of the cells could be "presented" by the modified cells and be recognized by the host as a "foreign" agent. Such recognition would trigger a rejection reaction, which could potentially render ineffective such treatments or, in severe cases, potentially cause auto-immune reactions.

[0006] More recently, the advent of genetic therapies has seen the substantial obstacle of immunogenicity of the viral vector utilized to administer the transgene, as well as the immunogenicity of the transgene protein itself after expression by the recipient's cells. The immunogenicity of vectors and transgenes results in: 1) diminished efficacy as vector and transgene they are bound and cleared by the antibodies generated by the recipient; 2) need for increased doses, which increase safety risks and costs; 3) difficulty or impossibility to re-dose if the subject develops antibodies against the vector or transgene after a prior dose. Sometimes, the recipients have pre-existing antibodies against the vector even before the first administration, due to cross-reaction with naturally-occurring viruses. Other therapies based on viruses (e.g., oncolytic viruses in cancer) and gene editing therapies (e.g., CRISPR-Cas9-based therapies), also suffer from immunogenicity.

[0007] As such, a need exists for methods and compositions for reducing immunogenicity induced by various biological therapeutics, including without limitation, antibodies, cell therapy, and gene therapy.

SUMMARY

[0008] In one aspect, the present disclosure provides a use of B cell inhibitor that is non-depletional in the manufacture of a pharmaceutical composition for reducing immunogenicity in a patient receiving or having received a biological therapeutic agent. Disclosed herein, in one ~~aspect~~example, is a method of reducing immunogenicity, comprising administering to a patient receiving or having received a biological therapeutic agent, an effective amount of B cell inhibitor that is non-depletional.

[0009] In some ~~embodiment~~examples, the biological therapeutic agent is selected from one or more of: gene therapy, gene editing therapy, messenger RNA (mRNA) therapy, oncolytic viruses, enzyme replacement therapy, antibody therapy, protein therapeutics, and cell therapy. In some

embodimentexamples, the biological therapeutic agent is gene therapy.

[0010] In some embodimentexamples, the B cell inhibitor is a CD32B×CD79B bi-specific antibody capable of immunospecifically binding an epitope of CD32B and an epitope of CD79B. In some embodimentexamples, the CD32B×CD79B bi-specific antibody comprises:

- (A) a VL_{CD32B} domain that comprises the amino acid sequence of SEQ ID NO: 1;
- (B) a VH_{CD32B} domain that comprises the amino acid sequence of SEQ ID NO: 2;
- (C) a VL_{CD79B} domain that comprises the amino acid sequence of SEQ ID NO: 3; and
- (D) a VH_{CD79B} domain that comprises the amino acid sequence of SEQ ID NO: 4.

[0011] In some embodimentexamples, the CD32B x CD79B bi-specific antibody is an Fc diabody comprising:

- (A) a first polypeptide chain that comprises the amino acid sequence of SEQ ID NO: 5;
- (B) a second polypeptide chain that comprises the amino acid sequence of SEQ ID NO: 6; and
- (C) a third polypeptide chain that comprises the amino acid sequence of SEQ ID NO: 7.

[0012] In some embodimentexamples, the method can further include administering the Fc diabody at a dose of between about 5 mg/kg and about 40 mg/kg, and at a dosage regimen of between one dose per 2 week and one dose per 6 weeks. In some embodimentexamples, the method can include administering the Fc diabody at a dose of about 10 mg/kg, and at a dosage regimen of one dose per 4 weeks. In some embodimentexamples, the method can include administering 3 doses of the Fc diabody at a dose of about 10 mg/kg at 2-6 week intervals.

[0013] In some embodimentexamples, the method can include administering a first dose about 2-6 weeks (e.g., 4 weeks) prior to administration of the biological therapeutic agent, a second dose at about the same time as administration of the biological therapeutic agent, and a third dose about 2-6 weeks (e.g., 4 weeks) after administration of the biological therapeutic agent.

[0014] In some embodimentexamples, the Fc diabody results in inhibition of its own immunogenicity upon administration, with lower prevalence and/or titers of anti-drug antibodies (ADA) at increased doses. In some embodimentexamples, the ADA does not neutralize the Fc diabody.

[0015] In some embodimentexamples, the Fc diabody, in a dose-dependent fashion, binds to at least 80% B cells upon administration, and remains bound to at least 50% of the B cells for at least 4 weeks after last administration.

[0016] In some embodimentexamples, the Fc diabody results in sustained inhibition of immunoglobulin production without depleting circulating B cells. In some embodimentexamples, the immunoglobulins include

one or more of IgM, IgA, IgG and IgE.

[0017] In some embodimentexamples, the method can further include monitoring the patient by examining the presence of specific antibodies against the biological therapeutic agent. In some embodimentexample, the method can further include administering one or more dose of the B cell inhibitor to further modulate immunogenicity.

[0018] In some embodimentexamples, the method can further include co-administering one or more immune-modulators, such as sirolimus, rapamycin, abatacept, teplizumab and immunoglobulin G-degrading enzyme of Streptococcus pyogenes.

[0019] Also provided herein are pharmaceutical compositions comprising the non-depletional B cell inhibitors disclosed herein, provided (e.g., packaged) at therapeutically effective unit doses. Instructions for dosage regimens as disclosed herein can also be provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] Figure 1: Schematic Overview of the Study.

[0021] Figures 2A-2C: Mean ($\pm$ SD) PRV-3279 Serum Concentrations (ng/mL) versus Time by Day on Linear Scale (Pharmacokinetic Population) (Figure 2A: Day 1, Figure 2B: Day 15, Figure 2C: Day 29).

[0022] Figures 3A-3C: Mean PRV-3279 Serum Concentrations (ng/mL) versus Time by Day on Semi-logarithmic Scale (Pharmacokinetic Population) (Figure 3A: Day 1, Figure 3B: Day 15, Figure 3C: Day 29).

[0023] Figures 4A-4B: Mean ($\pm$ SD) of PRV-3279 Serum Concentrations (ng/mL) versus Time (Day) by ADA Result by Dose (Pharmacokinetic Population) (Figure 4A: 3 mg/kg, Figure 4B: 10 mg/kg).

[0024] Figure 5: Box Plot of PRV-3279 Serum Pharmacokinetic Parameter by ADA Result by Dose (Pharmacokinetic Population).

[0025] Figure 6: Arithmetic Mean ($\pm$ SEM) of % MaxBinding of % anti-E/K+ (CD3-/CD19+) by Time and Treatment (Pharmacodynamic Population).

[0026] Figure 7: Arithmetic Mean ($\pm$ SEM) of Cell Numbers of B Cells (CD19+) - (cells/returned to μ L) (Pharmacodynamic Population).

[0027] Figure 8: Arithmetic Mean ($\pm$ SEM) of Reduction in Circulating Serum IgM Levels (Safety Population).

CLAIMS

1. ~~A method of reducing immunogenicity, comprising administering to a patient receiving or having received a biological therapeutic agent, an effective amount~~ Use of B cell inhibitor that is non-depletional in the manufacture of a pharmaceutical composition for reducing immunogenicity in a patient receiving or having received a biological therapeutic agent.
2. The ~~method~~use of claim 1, wherein the biological therapeutic agent is selected from one or more of: gene therapy, gene editing therapy, messenger RNA (mRNA) therapy, oncolytic viruses, enzyme replacement therapy, antibody therapy, protein therapeutics, and cell therapy.
3. The ~~use~~method of claim 1, wherein the biological therapeutic agent is gene therapy.
4. The ~~use~~method of any one of claims 1-3, wherein the B cell inhibitor is a CD32B×CD79B bi-specific antibody capable of immunospecifically binding an epitope of CD32B and an epitope of CD79B.
5. The ~~use~~method of claim 4, wherein the CD32B×CD79B bi-specific antibody comprises:
 - (A) a VL_{CD32B} domain that comprises the amino acid sequence of SEQ ID NO: 1;
 - (B) a VH_{CD32B} domain that comprises the amino acid sequence of SEQ ID NO: 2;
 - (C) a VL_{CD79B} domain that comprises the amino acid sequence of SEQ ID NO: 3; and
 - (D) a VH_{CD79B} domain that comprises the amino acid sequence of SEQ ID NO: 4.
6. The ~~use~~method of claim 4, wherein said CD32B x CD79B bi-specific antibody is an Fc diabody comprising:
 - (A) a first polypeptide chain that comprises the amino acid sequence of SEQ ID NO: 5;
 - (B) a second polypeptide chain that comprises the amino acid sequence of SEQ ID NO: 6; and
 - (C) a third polypeptide chain that comprises the amino acid sequence of SEQ ID NO: 7.
7. The ~~use~~method of claim 6, comprising ~~that~~administering the Fc diabody is to be administered at a dose of between ~~about~~ 5 mg/kg and ~~about~~ 40 mg/kg, and at a dosage regimen of between one dose per 2 week and one dose per 6 weeks.
8. The ~~method~~use of claim 6, comprising ~~administering that~~ the Fc diabody is to be administered at a dose of about 10 mg/kg, and at a dosage regimen of one dose per 4 weeks.
9. The ~~method~~use of claim 6, comprising ~~administering that~~ 3 doses of the Fc diabody are to be administered at a dose of about 10 mg/kg at 2-6 week intervals.
10. The ~~method~~use of claim 9, comprising ~~administering that~~ a first dose is to be administered

~~about~~ 2-6 weeks prior to administration of the biological therapeutic agent, a second dose is to be administered at about the same time as administration of the biological therapeutic agent, and a third dose is to be administered ~~about~~ 2-6 weeks after administration of the biological therapeutic agent.

11. The ~~method~~use of claim 6, wherein the Fc diabody results in inhibition of its own immunogenicity upon administration, with lower prevalence and/or titers of anti-drug antibodies (ADA) at increased doses.

12. The ~~method~~use of claim 11, wherein the ADA does not neutralize the Fc diabody.

13. The ~~method~~use of claim 6, wherein the Fc diabody, in a dose-dependent fashion, binds to at least 80% B cells upon administration, and remains bound to at least 50% of the B cells for at least 4 weeks after last administration.

14. The ~~method~~use of claim 6, wherein the Fc diabody results in sustained inhibition of immunoglobulin production without depleting circulating B cells.

15. The ~~method~~use of claim 14, wherein the immunoglobulin includes IgM, IgA, IgG and IgE.

16. The ~~method~~use of any one of claims 1-15, further comprising monitoring the patient by examining the presence of specific antibodies against the biological therapeutic agent.

17. The ~~method~~use of claim 16, further comprising ~~administering that~~ one or more dose of the B cell inhibitor is to be administered to further modulate immunogenicity.

18. The ~~method~~use of any one of claims 1-15 for use in combinatorial therapy, further comprising co-administering wherein said pharmaceutical composition is to be administered in combination with one or more immune-modulators.

19. The ~~method~~use of claim 18, wherein the one or more immune-modulators are selected from sirolimus, rapamycin, abatacept, teplizumab and immunoglobulin G-degrading enzyme of *Streptococcus pyogenes*.